GC-MS Detection and Quantification of Cocaine at Picogram Levels

Factors to consider when doing trace-level work

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Introduction

Not too long ago, I joined the Research Laboratory while it was still located in Washington, D.C., and my first assignment was to extract and quantify some particle standards that had been prepared at the Houston Advanced Research Center (HARC) for use as test targets in evaluating new drug detection equipment. These standards were either silanized glass beads or silver-coated nickel beads that had been spiked, respectively, with cocaine and heroin at nanogram levels. Little was I prepared for all the problems that I encountered in doing trace-level quantitative work, but I have gained a tremendous amount of knowledge in solving these problems, and this paper serves to share some of these findings. Results are presented for cocaine only, but similar observations were also made for heroin. New GC-MS operators can use this paper as a starting point for troubleshooting, and even experienced operators may benefit from applying some of the techniques that I have developed.

Experimental

Reagents and solution preparation

Initial method development used stock cocaine hydrochloride (COC) of minimum 90% purity. The internal standard (ISTD) was methyl docosanoate (Aldrich, CAS #929-77-1). About 1 mg each of COC and ISTD were weighed to the nearest 0.1 mg and dissolved together in 100.0 mL HPLC-grade $CHCl_3$ to make a 10 ng/μL stock solution of COC/ISTD. This stock solution was stored at 3°C in a Teflon-lined capped bottle, from which small portions were removed to make the analytical solutions. 10, 50, or 100 μL Drummond pipettes and 5 or 10 mL volumetric flasks were used to prepare picogram-level analytical solutions from the stock the same day as the analysis unless otherwise noted. Where appropriate, volumetric flasks were deactivated by overnight soaking in 1N HNO_3, followed by thorough rinsing with deionized water, 24-hour minimum soaking in 5% dimethyldichlorosilane (DMDCS, Supelco, 3-3065), thorough rinsing with toluene and methanol, and drying at 75°C.[1] Additionally, N,O-bis(trimethylsilyl) acetamide (BSA, Supelco, CAS #10416-59-8) was used to increase sensitivity and quantitative reproducibility as discussed below.

Solutions used to generate calibration curves for extraction analyses were made as follows. USP cocaine hydrochloride was dissolved in $CHCl_3$ to make a concentrated 1 μg/μL stock solution. This solution was diluted to a working 1 ng/μL solution, from which 1-mL analytical solutions of 5, 10, 30, 65, 100 pg/μL COC were made. Each analytical solution was spiked with 8 μL of 6 ng/μL ISTD solution and 2 μL BSA.

[1] This procedure was based on one for silylating liners found in an excellent reference book by Dean Rood, A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatographic Systems, Hüthig (1995).

GC-MS analysis

Analyses were done on Hewlett Packard (HP,) GC-MSD (5890/5970 or 6890/5973) operated in electron-impact (EI) mode with an ionization voltage of 70 eV and all other parameters set at autotune values. Several types of inlet liner were tried, but only a deactivated, dual-taper liner (*e.g.,* Supelco, 2-0485) gave good low-level sensitivity. 5% phenyl methyl silicone capillary columns from various manufacturers were used, all of which gave similar performance (J&W DB-5MS, 12 m x 0.2 mm x 0.32 μm; Supelco SPB-5 or Restek Rtx-5MS, 15 m x 0.25 mm x 0.25 μm). 2 μL splitless injections were made, and the purge valve was turned on after 0.75 min[2] with a 100 mL/min split vent flow. Inlet and transfer line temperatures were 250°C and 280°C, respectively. Oven temperature was held at 50°C for 0.50 min, ramped to 225°C at 50°C/min and held for 0.25 min, and finally programmed to 250°C at 25°C/min and held for 1.75 min. The carrier gas was 99.999% pure helium, and the gas line was equipped sequentially with moisture, hydrocarbon, and 2 oxygen traps (high capacity and indicating). In addition, a chemical trap was used in the carrier gas line between the mass flow controller and the inlet. A column head pressure of 7 psi gave a helium flow rate of 2.3 mL/min or a linear velocity of 79 cm/sec at 50°C.

Using the above temperature program, cocaine eluted at about 5.4 min and the ISTD at 6.2 min.[3] The mass detector was operated in the selected-ion monitoring (SIM) mode, detecting *m/z* 82, 182, and 303 for cocaine and 74, 87, and 354 for the ISTD. The dwell time for each ion was 100 ms, and quantification was done using the total ion chromatogram (TIC) for all selected ions rather than one single *m/z*.[4]

Results and Discussion

Part 1 – Pushing the limit

One problem associated with low level detection of any active compound is the possibility of loss due to adsorption on the walls of the container, before the solution even makes it into the GC inlet. To establish that this is not a concern for cocaine, two 20 pg/μL solutions of COC/ISTD were made and stored in four vials as follows:

(1) made in undeactivated volumetric flask and stored in a clear, undeactivated vial;
(2) made in DMDCS deactivated flask but stored in a clear, undeactivated vial;
(3) made in undeactivated flask but stored in an amber SilCote™ vial (Restek, 24640);
(4) made in DMDCS deactivated flask and stored in an amber SilCote™ vial.

After two days of storage at 3°C, no significant difference was observed in the cocaine signal among these four solutions (**Fig. 1**), indicating negligible loss due to adsorption on the walls of undeactivated glassware. Nevertheless, the cocaine signal, though quantifiable, was not sufficiently reproducible, and procedures had to be developed to deal with the sensitivity problems.

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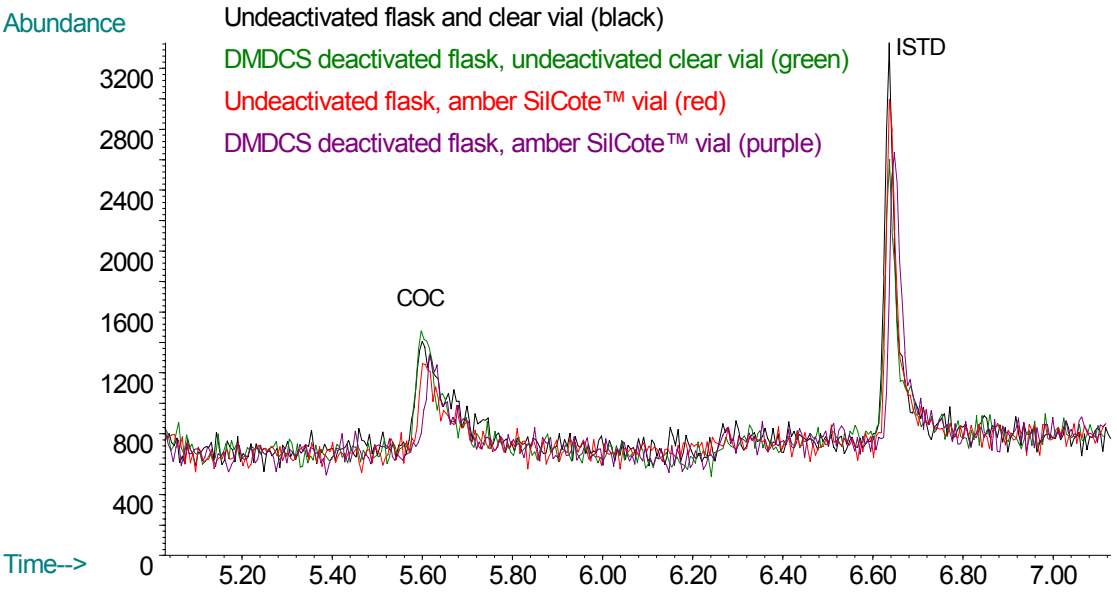
[2] At 2.3 mL/min flow rate and 0.75 min purge off time, the volume swept into the column is *ca.* 1700 μL or about twice the volume of the liner as recommended when doing splitless injection.

[3] The very fast temperature ramp lead to somewhat irreproducible retention times. Retention time reproducibility was verified by slowing down the temperature ramp for some runs, but a fast temperature ramp was used on a routine basis for shorter run times during method development.

[4] No difference in result was observed when using TIC *vs.* a single *m/z*, and it was a matter of convenience to quantify using the TIC.

Figure 1



As it turned out, the majority of the problems was inlet related, a fact that should not be surprising to experienced GC-MS operators, but some novel solutions were developed to deal with these problems. The first is an obvious one – clean the GC inlet. I recently attended an HP GC-MS operator's course in Atlanta, where the instructors suggested scrubbing the inlet with a brass wire brush, the type that is used to clean a 0.38-caliber gun barrel. I am a little wary of using such harsh treatment, since scrubbing can introduce additional active sites in the inlet, but I have scrubbed the inlet with solvent-dipped cotton swabs, followed by thorough solvent rinsing.[5] Real improvement was only observed, however, when I rinsed the inlet with BSA to passivate any active sites present, despite the fact that, ideally, the deactivated glass liner has sufficient volume to contain the vaporized sample. Excess BSA was gently baked away at 75°C[6] before reinstalling the split line and column.

As I have mentioned, the ideal situation is one in which all of the vaporized sample is contained inside the injection liner. This minimizes sample contact with hot metal surfaces, which can catalyze decomposition of thermally labile compounds. In addition, liner overloading results in backflash in which some of the sample condenses outside the liner, in the split or purge lines. Consequently, it becomes very difficult to get reproducible quantitative results. Using a dual-taper liner helps address both of these problems. At 7 psi and 250°C, a 2-μL $CHCl_3$ liquid injection will vaporize to a volume of 714 μL (see Appendix), whereas the liner volume is approximately 900 μL and the tapers provide additional containment of the vapor plume.

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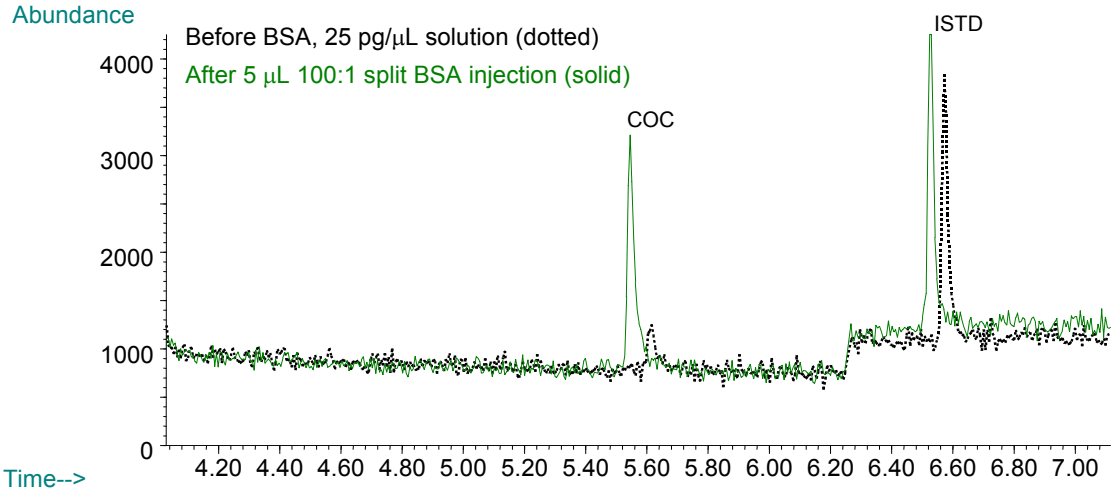
[5] Two notes of caution – the inlet can be scrubbed with a wire brush only a limited number of times before needing replacement, and the split line and column must be removed before any solvent is introduced into the inlet for cleaning. Typically, it is recommended to start cleaning with a polar solvent and move progressively to a nonpolar solvent. I have used methanol, acetone, and chloroform in succession with good results.

[6] A higher temperature will cause a breakdown of the passive layer. By the same token, the inlet should be cooled to 75°C or below before installing a new liner and the system allowed to purge at the low temperature for at least 10 minutes before heating or the moist heat will cause the liner to become deactivated.

Despite the fact that the liners are supposed to be deactivated, I have found substantially improved sensitivity by injecting BSA directly into the GC, effectively doing an in-situ deactivation of any remaining active sites. This effect is observed even when using a new (unused) liner and a new column, so that there is no question of active sites having arisen from contamination or column degradation. **Figure 2** compares chromatograms for a 25 pg/μL solution of COC/ISTD. Whereas the cocaine signal is barely detectable above the background before BSA deactivation, the signal is nearly as big as the ISTD signal following a 5 μL BSA injection into the hot inlet. Notice that the ISTD signal is comparable between the two injections, indicating that methyl docosanoate, as would be expected, is relatively inert and unreactive even in a hot injection port.

Figure 2



Carrying the deactivation idea one step further, I added[7] BSA directly to the analytical solution prior to making it to volume and observed a nearly tenfold increase in the cocaine S/N for a 10 pg/μL solution (**Fig. 3**). A question that immediately comes to mind is whether BSA has somehow lowered the cocaine limit of detection and quantitation (LOD/LOQ) or whether it has simply derivatized contaminants which then coeluted with and increased the cocaine signal. The latter scenario is an unlikely possibility for several reasons. It requires that the contaminants be something that can be quickly derivatized under ambient conditions, since the solutions were not heated and were analyzed almost immediately following preparation. It requires that the contaminants coelute with cocaine and have the same mass fragments (82, 182, 303) in the same ratio as cocaine. Some cocaine impurities and decomposition products such as ecgonine and benzoylecgonine can be derivatized by BSA, but they would have different retention times as well as mass fragment ratios. Finally, the amount of impurity present in the solution was not sufficient to give the observed increase in cocaine S/N. To verify further that contamination is not an issue, injections of BSA-spiked solvent, $CHCl_3$ in this case, were made both before and after BSA-spiked COC/ISTD injections (**Fig. 4**). The first solvent injection gave a clean, flat baseline. After six injections of a 10 pg/μL COC/ISTD

[7] I used 5 μL BSA to 10 mL analytical solution, although any amount from 2 to 10 μL seemed to work equally well. Larger amounts of BSA can sometimes cause junk peaks to appear as the BSA starts to react with both the vial and inlet septa.

solution, in which strong cocaine and ISTD signals were observed, another solvent injection was made, and the resulting chromatogram was still clean.

Figure 3

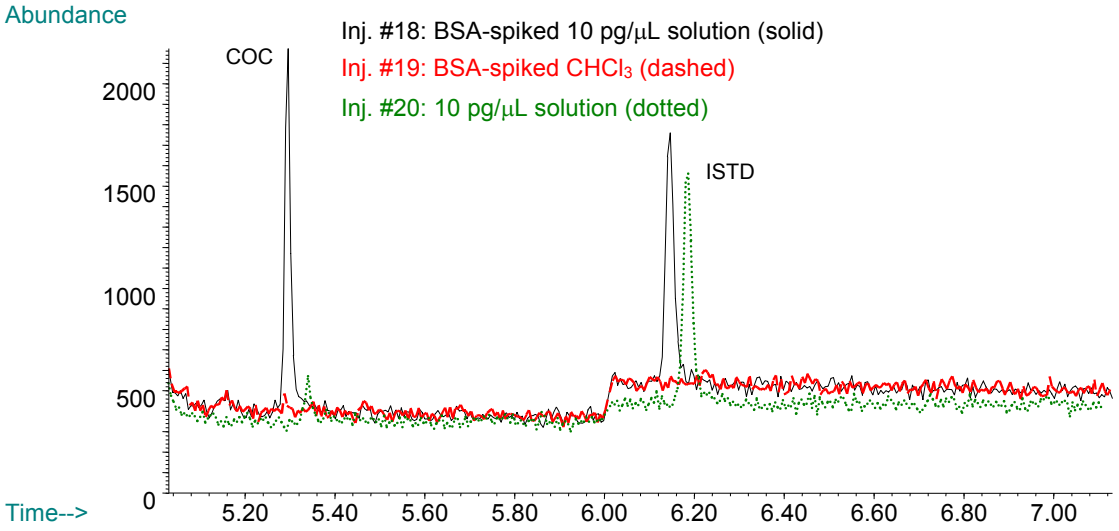
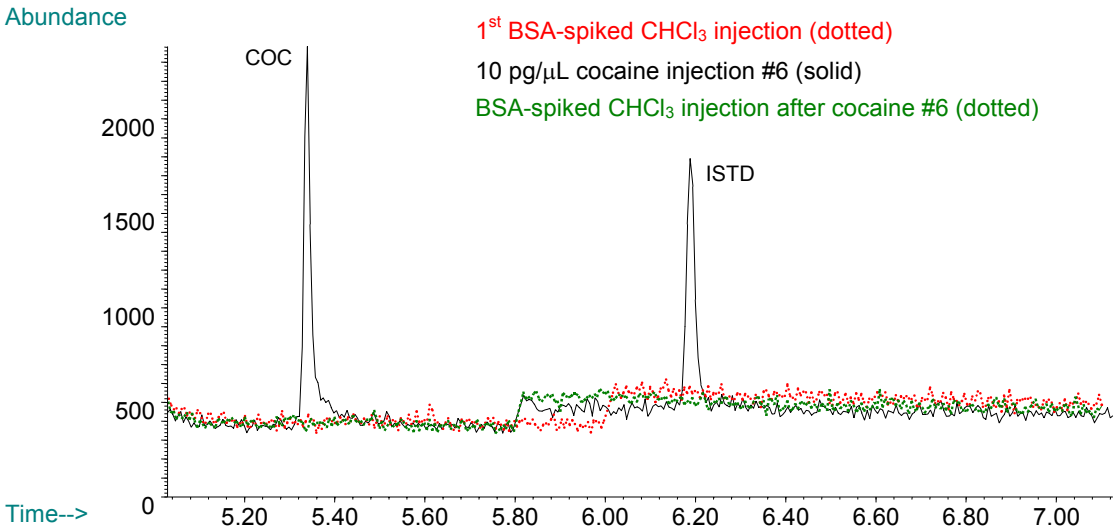


Figure 4

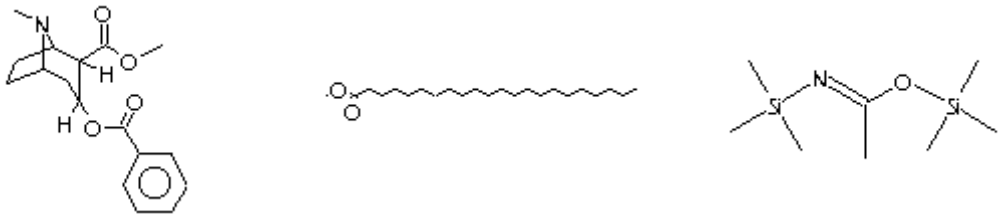


Since cocaine itself cannot be derivatized (**Fig. 5**), it appears that BSA has lowered the cocaine LOD/LOQ by suppressing breakdown of cocaine *in the hot inlet*.[8] The ISTD signal, on the other hand, remained relatively unchanged between the BSA-spiked solution and one without BSA, reflecting once again the thermal stability of the fatty acid methyl esters. As I mentioned in the

[8] A useful compound for monitoring the thermal degradation of cocaine is anhydroecgonine methyl ester (*methyl ecgonidine, EDME*), with major mass fragments 181 (M+) and 152 (most abundant).

introduction, similar increase in sensitivity was obtained for heroin, where BSA suppresses the thermal degradation of heroin to 6-monoacetyl morphine. Breakdown of both cocaine and heroin is catalyzed by the presence of active sites in the inlet, especially when methanol is used as a solvent. In one instance, I have seen pharmaceutical-grade heroin analyzed as only 70% pure because it was dissolved in methanol and injected into an inlet containing a cracked liner. Had a standard been run using the internal standard method of quantification, the degradation would have been immediately apparent and corrective measures been taken before proceeding with the analysis.

Figure 5

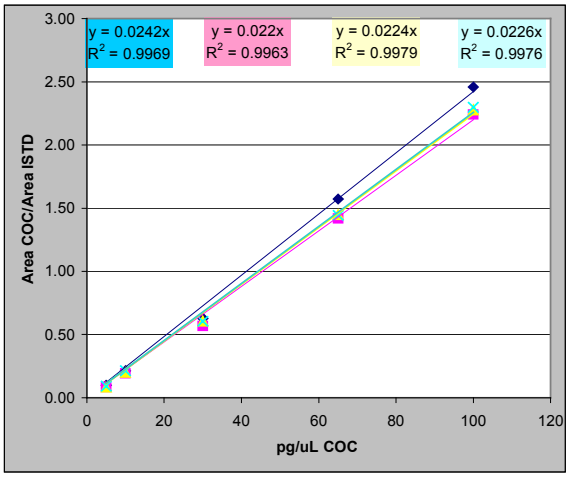


cocaine methyl docosanoate BSA

Part 2 – Some quantitative results

All of the work discussed above was done on the old 5890/5970 GC/MSD, initially without the benefit of an autosampler (ALS). Even at low nanogram levels, manual injections could not give quantitative results with reproducibility better than 20% relative standard deviation (RSD). With an ALS, quantitative reproducibility was much better, but the LOQ was still not quite low enough for the work at hand. With the new 6890/5973, I have been able on a routine basis to quantify cocaine at 5 pg/μL and heroin at 10 pg/μL. Five-point calibration curves have been generated for both drugs from the lower limit to 100 pg/μL with better than 0.99 correlation. **Figure 6** gives an example of four calibration curves for cocaine, which remained relatively unchanged by the extraction samples that were run in between each calibration set.

Figure 6

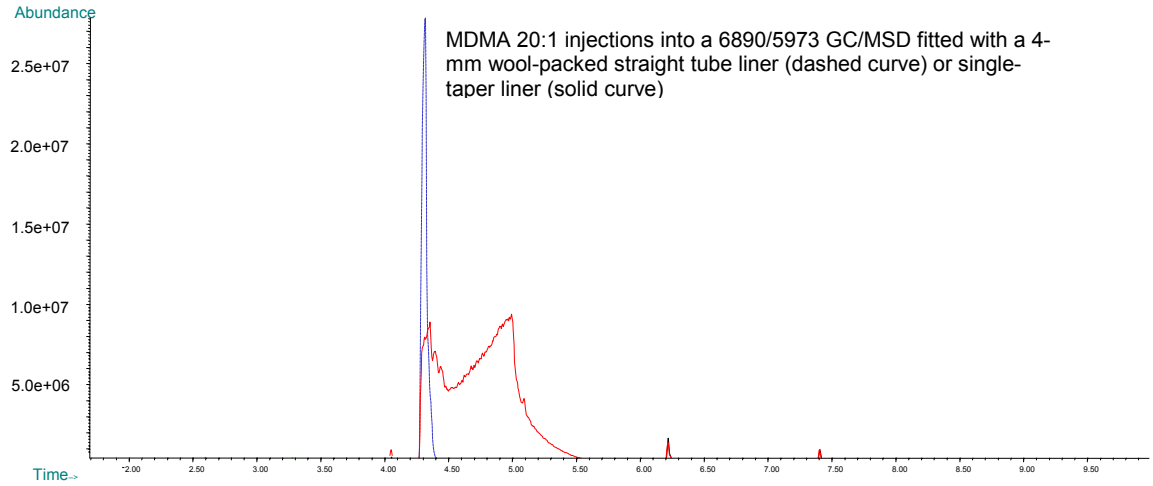


Part 3 – Other experimental pointers

Throughout this paper, I have stressed the importance of good inlet maintenance for doing trace-level quantitative work, but many of these procedures are equally useful when doing qualitative work. I have mentioned the degradation problems that might be encountered when using methanol as a solvent. Methanol solutions will also often give periodic ghost peaks after repeated injections through a septum-sealed vial. These peaks may be misinterpreted as inlet septum bleed or, worse yet, column bleed. They arise, however, from the sample vial cap[9] – once the Teflon lining has been punctured, methanol comes into contact with the outer methyl silicone layer of the cap and causes methyl silicone to leach into the solution. These warnings do not mean methanol should never be used as a solvent; it is very useful for extracting most drugs and even some excipients and is, therefore, a good solvent for identification work. It should not, however, be used in quantitative analyses.

Another factor that will affect quantitative results is the choice of inlet liner. Only the dual-taper liner works well for trace-level work. I have tried the single-taper liner and have found it to give noisier baselines, and hence lower S/N, than the dual-taper one. The single-taper liner does not work well for split injections either, giving very poor peak shapes, which would in turn give unreliable quantitative data. **Figure 7** compares two chromatograms that were generated from split injections of an MDMA (3,4-methylenedioxymethamphetamine) sample. Good peak shape was obtained only when a straight-tube liner (Supelco, 2-0486) was used.

Figure 7



Conclusion

Having been a full-time GC-MS operator for only a few years, I dare not claim to know all there is to know about running, maintaining and troubleshooting a GC-MS system. Nevertheless, I have had to come up with some unusual solutions to push the limits of the instrument, and I have learned along the way some basic facts that are worth remembering when troubleshooting. This paper shares my experiences in hopes of saving some other chemists the need to reinvent the wheel.

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[9] Septum bleed from the vial also occurs when using BSA-spiked $CHCl_3$, since BSA is very reactive and will readily cause leaching of methyl silicone in into the solution. To get around this problem, I leave the vials uncapped. Quantitative results are unaffected because of the use of an internal standard, and filling a vial about 2/3 full will give enough solution to last the course of the day.

Appendix

Ideal gas equation used to calculate the expansion volume of a liquid injection into a GC inlet.

$$V = \frac{nRT}{P} = 10^3 \frac{(vd/M)RT}{(p * 0.068) + 1}$$

where V = volume of vapor in μL
n = number of moles of vapor
R = ideal gas constant, 0.0821 L-atm/mol-K
T = temperature of inlet (°C + 273), K
P = pressure of vapor, atm
v = volume of liquid injected, μL
d = density of liquid, mg/mL
M = molecular mass of liquid, g/mol
p = column head pressure, psi
0.068 psi/atm and a standard pressure of 1 atm